IdentiClone®

Instructions for Use

CE IVD

IdentiClone® T-Cell Receptor Gamma Gene Rearrangement Assay 2.0

For identification of T cell clonality.

IVD For *In Vitro* Diagnostic Use

invivoscribe

Storage Conditions: **-85°C to -65°C**
(DNA controls may be separated from assay kits and stored at 2°C to 8°C)

Catalog#	Products	Quantity
REF 9-207-0101	IdentiClone T Cell Receptor Gamma Gene Rearrangement Assay 2.0	33 Reactions
REF 9-207-0111	IdentiClone T Cell Receptor Gamma Gene Rearrangement Assay 2.0 MegaKit	330 Reactions

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1. Intended Use

The IdentiClone T Cell Receptor Gamma Gene Rearrangement Assay 2.0 is an in vitro diagnostic product intended for PCR-based detection of clonal T-cell receptor gamma chain gene rearrangements in patients with suspect lymphoproliferations.

Specifically, the T Cell Receptor Gamma Gene Rearrangement Assay 2.0 can be used to identify clonality in suspect lymphoproliferations.

2. Summary and Explanation of the Test

2.1. Background

Rearrangements of the antigen receptor genes occur during ontogeny in B and T lymphocytes and generate products that are unique in length and sequence. Polymerase chain reaction (PCR) assays can be used to identify lymphocyte populations derived from a single cell by detecting the unique V-J gene rearrangements present within these antigen receptor loci.[1] This IdentiClone PCR assay employs multiple consensus DNA primers that target conserved genetic regions within the T cell receptor gamma chain gene and amplify the region with fluorescently labeled primers, followed by fractionation by capillary electrophoresis and analysis by instrument software. This DNA based test is used to detect the vast majority of clonal T-cell populations. Presence or absence of clonality can support the differential diagnosis of reactive lesions and certain T and B cell malignancies.

This assay cannot reliably detect clonality present at less than 5% of the total lymphocyte population. Always interpret the results of molecular clonality testing in the context of all available clinical, histological and immunophenotypic data.

2.2. Summary

This test kit consists of a single master mix that contains primers that target the Vγ2, 3, 4, 5, 8, 9, 10, and 11 and Jγ1/Jγ2, JγP, and JγP1/JγP2 regions, generating PCR amplicons with an expected size range between 159 and 207 nucleotides (nt). The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 nucleotides to ensure that the quality and quantity of input DNA is adequate to yield a valid result. The procedure uses a single thermal cycler program and similar detection methodology for all Invivoscribe Gene Clonality Assays, which improves consistency and facilitates cross training on a broad range of assays.

Peak analysis is supported by a software based algorithm that calculates the relative peak height ratio (RPR) and a statistical parameter D(x) value for each peak. The RPR is calculated by dividing the height of each peak to the smaller of its neighboring peaks and it must exceed a cutoff of 4.0. The D(x) value is based on a variation of the Kolmogorov-Smirnov test, which compares two empirical distributions and determines whether they are statistically different and its value must be greater than 0.0419.

This assay was developed by Invivoscribe. The performance of this assay was reviewed and validated by the EuroClonality/BIOMED-2 Group. Euroclonality manuscript in preparation: multicenter study with 250 clinical patient specimens.

3. Principles of the Procedure

3.1. Polymerase Chain Reaction (PCR)

PCR assays are routinely used for the identification of clonal T-cell populations. This test amplifies the DNA between primers that target conserved regions within the variable (V) and the joining (J) regions that flank the unique hypervariable antigen-binding region 3 (CDR3). These conserved regions lie on either side of an area within the V-J region where programmed genetic rearrangements occur during maturation of all B and T lymphocytes. The antigen receptor genes that undergo rearrangement are the immunoglobulin heavy chain and light chains in B-cells, and the T cell receptor genes in T- cells. Each B- and T-cell has a single productive V-J rearrangement that is unique in both length and sequence. Therefore, when DNA from a normal or polyclonal population is amplified using primers that flank the V-J region, a Gaussian distribution (bell-shaped curve) of amplicon products within an expected size range is generated. This Gaussian distribution reflects the heterogeneous population of V-J rearrangements. (In certain cases, where lymphocyte DNA is not present, no product is detected.) DNA from samples containing a clonal population yield one or two prominent amplified products (amplicons) within a diminished polyclonal background.

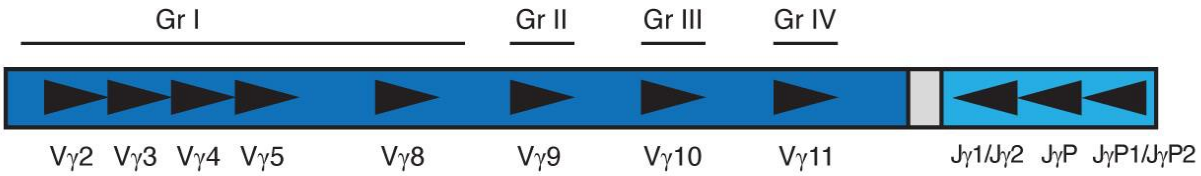

Figure 1. This diagram of the T cell receptor gamma gene shows the approximate placement of the upstream and downstream DNA primers.

Since the antigen receptor genes are polymorphic (consisting of a heterogeneous population of related DNA sequences), it is difficult to employ a single set of DNA primer sequences to target all of the conserved flanking regions around the V-J rearrangement. N-region diversity, and somatic mutation further scramble the DNA sequences in these regions. Therefore, a multiplex master mix, which targets multiple V and J regions (Figure 1), is required to detect the majority of clonal rearrangements. As indicated, clonal rearrangements are identified as one or two prominent, single-sized products within the background of different-sized amplicon products that form the Gaussian distribution around a statistically favored, average-sized rearrangement.

3.2. Fluorescence Detection

Fluorescence detection is commonly used to resolve the different-sized amplicon products using a capillary electrophoresis instrument. Primers are conjugated with a 6FAM fluorescent dye (fluorophore) so that they can be detected after excitation by a laser in the capillary electrophoresis instrument. This highly sensitive detection system provides single nucleotide size resolution and relative quantification. Inter and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 2 nucleotides. This reproducibility and sensitivity coupled with the automatic archiving of specimen data allows for the monitoring, tracking, and comparison of data from individual patients over time.

4. Reagents

4.1. Reagent Components

Table 1. Available Kits

Catalog #	Description	Quantity
REF 92070101	IdentiClone T-Cell Receptor Gamma Gene Rearrangement Assay 2.0	33 Reactions
REF 92070111	IdentiClone T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 MegaKit	330 Reactions

Table 2. Reagent Components

Reagent	Catalog #	Reagent Components (active ingredients)	Unit Quantity	92070101 # of Units	92070111 # of Units	Storage Temp.
Master Mixes	22070091	***TCRG* – 6FAM** Multiple oligonucleotides targeting the Vγ2, 3, 4, 5, 8, 9, 10, & 11 and Jγ1/Jγ2, JγP, and JγP1/JγP2 regions of the T cell receptor gamma gene in a buffered salt solution.	1500 µL	1	10	-85°C to -65°C
Template Amplification Control	20960021	**Specimen Control Size Ladder – 6FAM** Multiple oligonucleotides targeting housekeeping genes.	1500 µL	1	10	
Positive Control DNA	40883320	**5% *TCRG* Positive Control DNA** 50 µg/mL of DNA in 1/10th TE solution	50 µL	1	5	2°C to 8°C or -85°C to -65°C
Negative (Normal) Control DNA	40920020	***TCRG* Negative Control DNA** 50 µg/mL of DNA in 1/10th TE solution	50 µL	1	5	

Note: There are no preservatives used in the manufacture of this kit.

4.2. Warnings and Precautions

- IVD **This Product is for *in vitro* diagnostic use**.
- Use this assay kit as a system. Do not substitute other manufacturer's reagents. Dilution, reduction of amplification reaction volumes, or other deviation in this protocol may affect the performance of this test and/or nullify any limited sublicense that comes with the purchase of this testing kit.
- Materials are stable until the labeled expiration date when stored and handled as directed. Do not use kits beyond their expiration date.
- Close adherence to the protocol will assure optimal performance and reproducibility. Use care to ensure use of correct thermal cycler program, as other programs may provide inaccurate/faulty data, such as false positive and false negative results.
- Do not mix or combine reagents from kits with different lot numbers.
- Wear appropriate personal protective equipment and follow good laboratory practices and universal precautions when working with specimens. Handle specimens in approved biological safety containment facilities and open only in certified biological safety cabinets. Use glass distilled de-ionized molecular biology grade water for the preparation of specimen DNA.
- Due to the high analytical sensitivity of this test, use extreme care to avoid the contamination of reagents or amplification mixtures with samples, controls or amplified materials. Closely monitor all reagents for signs of contamination (*e.g.,* negative controls giving positive signals). Discard reagents suspected of contamination.
- To minimize contamination, wear clean gloves when handling samples and reagents and routinely clean work areas and pipettes prior to doing PCR.
- Autoclaving does not eliminate DNA contamination. Always follow uni-directional work flow in the PCR laboratory; begin with master mix preparation, move to specimen preparation, then to amplification, and finally to detection. Do not bring amplified DNA into the areas designated for master mix or specimen preparation.
- Dedicate all pipettes, pipette tips, and any equipment used in a particular area to that area of the laboratory.
- Use sterile, disposable plastic ware whenever possible to avoid RNase, DNase, or cross-contamination

4.4. Storage and Handling

- For any duration other than immediate use, **store assay kits at -65ºC to -85ºC**.
- The optimum storage temperature for the DNA controls is 2ºC to 8ºC, but DNA controls can also be stored at -85ºC to -65ºC.
- All reagents and controls must be thawed and vortexed or mixed thoroughly prior to use to ensure that they are mixed completely. Excessive vortexing may cause labeled primers to lose their fluorophores.
- Materials are stable until the labeled expiration date when stored and handled as directed. Do not use kits beyond their expiration date.
- The PCR master mixes and controls have been validated for 6 freeze-thaw cycles with no loss in performance. Aliquot reagents into sterile o-ring screw-cap tubes if more freeze-thaw cycles are necessary.

5. Instruments

5.1. Thermal cycler

- Use or Function: Amplification of DNA samples
- Performance Characteristics and Specification:
  - Minimum Thermal Range: 15 ºC to 96 ºC
  - Minimum Ramping Speed: 0.8 ºC/sec
- Follow manufacturer's installation, operation, calibration, and maintenance procedures.
- See section 7.4: *Amplification* for thermal cycler program.

5.2. ABI Capillary Electrophoresis Instruments

- Use or Function: Fragment detection and analysis
- Performance Characteristics and Specification:
  - The following capillary electrophoresis instruments will meet the performance needs for this assay:
    - ABI 3100 Avant Genetic Analyzer (4-capillaries)
    - ABI 3100 Genetic Analyzer (16-capillaries)
    - ABI 3130 Genetic Analyzer (4-capillaries)
    - ABI 3130XL Genetic Analyzer (16-capillaries)
    - ABI 3500XL Genetic Analyzer (24-capillaries)
- Follow manufacturer's installation, operation, calibration, and maintenance procedures.
- The ABI instrument used must be calibrated with appropriate Matrix Standards as outlined in section 7.2: *Materials Required but Not Provided*
- Use the default settings for your polymer and capillary type.
- See section 7.5: *ABI Fluorescence Detection* for details.

*Warning: These are not CE marked products

6. Specimen Collection and Preparation

6.1. Precautions

Biological specimens from humans may contain potentially infectious materials. Handle all specimens in accordance with the OSHA Standard on Bloodborne Pathogens or Biosafety Level 2.

6.2. Interfering Substances

The following substances are known to interfere with PCR:

- Divalent cation chelators
- Low retention pipette tips
- EDTA
- Heparin

6.3. Specimen Requirements and Handling

This assay tests genomic DNA extracted and purified from peripheral blood, bone marrow aspirates or paraffin embedded tissue.

6.4. Sample Preparation

Extract the genomic DNA from patient specimens as soon as possible. Resuspend DNA to a final concentration of 10 µg to 200 µg per mL in 1/10th TE (1 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) or in molecular biology grade or USP water. This is a robust assay system. A wide range of DNA concentrations will generate a valid result. Therefore, quantifying and adjusting DNA concentrations is generally not necessary. Testing sample DNA with the Specimen Control Size Ladder master mix will ensure that DNA of sufficient quality and quantity was present to yield a valid result.

6.5. Sample Storage

Store genomic DNA at 2ºC to 8ºC or at -85ºC to -65ºC for long term storage.

7. Assay Procedure

7.1. Materials Provided

Table 3. Materials Provided.

Catalog #	Description
22070091CE	*TCRG* – 6FAM
20960021	Specimen Control Size Ladder – 6FAM
40883320	5% *TCRG* Positive Control DNA
40920020	*TCRG* Negative Control DNA

7.2. Materials Required (not provided)

Table 4. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Catalog #	Notes
DNA Polymerase	Roche: • EagleTaq DNA Polymerase Invivoscribe, Inc.: • EagleTaq DNA Polymerase[1] or equivalent	5206944190 60980100	N/A
Glass Distilled De-ionized Molecular Biology Grade or USP	N/A	N/A	Water must be sterile and free of DNase and RNase.
Calibrated Pipettes	N/A	N/A	Must be able to accurately measure volumes between 1 µL and 1000 µL
Thermal cycler	N/A	N/A	N/A
Vortex Mixer	N/A	N/A	N/A
PCR plates or tubes	N/A	N/A	Sterile
Filter barrier pipette tips	N/A	N/A	Sterile, RNase/DNase/Pyrogen-free
Microcentrifuge tubes	N/A	N/A	Sterile
Hi-Di Formamide	Invivoscribe, Inc.: • HI-Deionized Formamide Applied Biosystems: • Hi-Di™ Formamide	60980041 4311320	N/A
ABI Capillary Electrophoresis Instrument	Applied Biosystems: • ABI 3100, 3130, or 3500 series	N/A	N/A

Size Standards	Invivoscribe, Inc.:
	• Hi-Di Formamide w/ROX size standards for ABI 3100
	Applied Biosystems:
	• For ABI 3100 or 3130 instruments:
	  ○ GeneScan™ - 400HD [ROX]™
	• For ABI 3500 instruments:
	  ○ GeneScan - 600 [LIZ]™ v2.0
		60980061
		
		402985
		4408399
			N/A

Spectral Calibration Dye Sets	Applied Biosystems:
	• For ABI 3100 and 3130 instruments:
	  ○ DS-30 Matrix Standard Kit (Dye Set D)
	• For ABI 3500 instruments:
	  ○ DS-33 Matrix Standard Kit (Dye Set G5)
		4345827
		
		4345833
			N/A

Polymer	Applied Biosystems: • POP-7 Polymer: ○ POP-7™ for 3130/3130XL/3500XL Genetic Analyzers	4352759	N/A

[1]**Note:** This product is for sale and use in the European Economic Area only. It is not to be resold or transferred to another party. See also Legal Notice in section 15.

7.3. Reagent Preparation

- All samples can be tested using the Specimen Control Size Ladder master mix to ensure that no inhibitors of amplification are present and there is DNA of sufficient quality and quantity to generate a valid result.
- Test samples in duplicate. If duplicate testing provides inconsistent results, re-testing or re-evaluation of the sample is necessary.
- Positive, negative, and no template controls must be tested.

7.3.1. Using gloved hands, remove the master mixes from the freezer. Allow the tubes to thaw completely; then gently vortex to mix.

7.3.2. Remove the calculated volume of each master mix to individual microcentrifuge tubes.

- The aliquot volume is 45 µL for each reaction.
- Add 15% overage to ensure an adequate volume is available.
- For the *TCRG* – 6FAM master mix, the number of reactions (**n**) is:

n = 2 × # of samples	(Run each sample in duplicate)
+ 1	5% *TCRG* Positive Control DNA
+ 1	*TCRG* Negative Control DNA
+ 1	No Template Control (water)
+ 1	Additional Reaction
n = 2 × # of samples + 4	**Total**

- The total aliquot volume for the *TCRG* – 6FAM master mix is **n × 45µL**.
- For the Specimen Control Size Ladder master mix, the number of reactions (**m**) is:

m = # of samples	(Run each sample in singlicate)
+ 1	*TCRG* Negative Control DNA
+ 1	No Template Control (water)
+ 1	Additional Reaction
m = # of samples + 3	**Total**

- The total aliquot volume for the Specimen Control Size Ladder master mix is **m × 45 µL**.

7.3.3. Add 1.25 units (or 0.25 µL @5 U/µL) of Taq DNA polymerase per reaction to each master mix.

- Add **n × 0.25 µL** Taq DNA polymerase to the *TCRG* master mix and **m × 0.25 µL** Taq DNA polymerase to the Specimen Control Size Ladder master mix. Gently vortex to mix.

7.3.4. For each reaction, aliquot 45 µL of the appropriate master mix + DNA polymerase solution into individual wells in a PCR plate or tube.

7.3.5. Add 5 µL of appropriate template (sample DNA, positive control DNA, negative control DNA or water) to the individual wells containing the respective master mix solutions. Pipette up and down several times to mix.

7.3.6. Cap or cover the PCR plate.

- Samples are now ready to be amplified on a thermal cycler.

Quick Guide

For each master mix and **n** reactions, mix:

n × 45 µL	Master Mix
n × 0.25 µL	Taq DNA polymerase

Vortex gently to mix.

Aliquot **45 µL** of master mix + DNA polymerase solution into each reaction well.

Add **5 µL** of appropriate Template to each well.

Total reaction volume =	**50 µL**

7.4. Amplification

7.4.1. Amplify the samples using the following PCR program:

- Use the **calculated** option for temperature measurement with the BioRad MJ Research PTC thermal cyclers.

Table 5. Thermal cycling conditions

Step	Temperature	Duration	Cycle
1	95°C	7 minutes	1
2	95°C	45 seconds	35
3	60°C	45 seconds	
4	72°C	90 seconds	
5	72°C	10 minutes	1
6	15°C	∞	1

7.4.2. Remove the amplification plate or tubes from the thermal cycler.

- Although amplified DNA is stable at room temperature for extended periods of time, store PCR products at 2ºC to 8ºC until detection.
- Detection must be performed within 30 days of amplification.

7.5. ABI Fluorescence Detection

Please note that for ABI fluorescence detection a preceding peak is often seen and is an artifact due to the detection method the ABI platforms use. Preceding peaks are sometimes skewed and have bases that slope on the right side towards the real peak. This is especially evident in the Specimen Control Size Ladder master mix where the 96 nucleotide (nt) peak has a preceding peak that shows up at 84 nt.

ABI 3100 and 3130 Platforms:

7.5.1. In a new microcentrifuge tube, mix an appropriate amount (10 µL per reaction) of Hi-Di Formamide with ROX Size Standards. Vortex well.

7.5.2. In a new 96-well PCR plate, add 10 µL of Hi-Di Formamide with ROX size standards to individual wells for each reaction.

7.5.3. Transfer 1 µL of each reaction to the wells containing Hi-Di Formamide with ROX size standards.

- Add only one sample per well.
- Pipette up and down to mix.

7.5.4. Cap or cover the PCR plate.

7.5.5. Heat denature the samples at 95ºC for 2 minutes, then snap chill on ice for 5 minutes.

7.5.6. Prepare a **sample sheet** and **injection list** for the samples.

7.5.7. Run the samples on an ABI 3100/3130 capillary electrophoresis instrument according to its user manual.

- Data are automatically displayed as size and color specific peaks.

7.5.8. Review profile and controls, report results. (See sections 8: *Interpretation of Results* and 10: *Expected Values*)

ABI 3500 Platforms:

7.5.9. In a new microcentrifuge tube, mix an appropriate amount (9.5 µL per reaction) of Hi-Di Formamide with LIZ Size Standards. Vortex well.

7.5.10. In a new 96-well PCR plate, add 9.5 µL of Hi-Di Formamide with LIZ size standards to individual wells for each reaction.

7.5.11. Transfer 0.5 µL of each reaction to the wells containing Hi-Di Formamide with LIZ size standards.

- Add only one sample per well.
- Pipette up and down to mix.

7.5.12. Cap or cover the PCR plate.

7.5.13. Heat denature the samples at 95ºC for 3 minutes, then snap chill on ice for 5 minutes.

7.5.14. Prepare a **sample sheet** and **injection list** for the samples.

7.5.15. Run the samples on an ABI 3500 capillary electrophoresis instrument according to its user manual.

- Data are automatically displayed as size and color specific peaks.

7.5.16. Review profile and controls, report results. (See sections 8: *Interpretation of Results* and 10: *Expected Values*)

7.6. Data Analysis

The *TCRG* Algorithm worksheet has been developed to analyze the *TCRG* V2 data output.

7.6.1. Open the ***TCRG* Algorithm Worksheet** (the worksheet requires Microsoft Excel).

7.6.2. Add raw data files derived from CE analysis to a new project in *GeneMapper* software.

7.6.3. Verify that **Analysis Method** selected is *Microsatellite Default* and that the appropriate *Size Standard* is selected.

- It may be necessary to lower the *Minimum Peak Height* threshold in order to detect all peaks in a Gaussian distribution:
  - select **GeneMapper Manager** from the *Tools* menu, go to the **Analysis Methods** tab, open the *Microsatellite Default* **Analysis Method Editor**.
  - Go to the *Peak Detector* tab, select **User Specified (rfu)** toggle, and input the desired peak height for the *Blue* dye.

7.6.4. In the *Analysis* menu, select **Analyze**.

7.6.5. For each analyzed sample file, open the associated display plot.

7.6.6. To ensure that only the *Blue Dye* is shown in the display plot, go to the **View** menu, select **Dyes → Blue Dye**.

7.6.7. Next, in the **View** menu, choose **Tables → Sizing Table**.

- Highlight the display plot peaks in the valid size range from *159 nt to 207 nt*.

7.6.8. In the *Sizing Table*, copy the **Size (nt)** and **Height (RFU)** column data for the highlighted peaks within the valid size range.

7.6.9. Paste the peak size and height data into the unlocked portion of the *TCRG* Algorithm worksheet (cells are highlighted in grey).

- GeneMapper version 3.5 and lower, requires this data to be entered manually into the worksheet.

7.6.10. The worksheet will output a summary of *RPR*, *D(x)*, and *%RFU (max)* for the five peaks that are the most significant outliers from a normal Gaussian distribution.

- If a peak in the summary table meets the criteria for a clonal peak as defined in the *TCRG* Algorithm worksheet, it will read **Yes** in the column titled *Significant?*.
- If a peak in the summary table does not meet the criteria for a clonal peak as defined in the *TCRG* Algorithm worksheet, it will read **No** in the column titled *Significant?*.

7.6.11. The following criteria, as well as those implemented in the worksheet, define peaks as **Positive for Clonality**:

- Analysis using the worksheet must be accompanied by visual confirmation that the worksheet is interpreting the sample correctly.
- Non-clonal samples feature a polyclonal background approximating a Gaussian distribution of peaks within the valid size range. Clonal samples generally feature a polyclonal background with a Gaussian distribution within the valid size range, from which a suspected clonal peak emerges as an outlier from the Gaussian distribution.
- Shoulder peaks may accompany suspected clonal peaks. A shoulder peak is defined as a peak 1 nt upstream or downstream in relation to a suspected clonal peak: whose height is lower than that of the neighboring suspected significant peak, but higher than the background distribution. Shoulder peaks may also be connected to suspected clonal peaks above the baseline. Shoulder peaks are generally considered background and should not be evaluated as suspected clonal peaks.
- The *D(x)* value of the suspected clonal peak, as calculated within the locked portion of the worksheet, must be **≥ 0.0419**.
- The *RPR* of the suspected clonal peak (calculated by dividing the suspected clonal peak height by the peak height of the smaller of its neighboring peaks) must be **≥ 4.0X**.
- The *RFU* of the suspected clonal peak must be **≥ 20%** of the RFU of the highest peak in that sample.
- If the suspected clonal peak meets these criteria (flagged by the worksheet as "Significant"), the peak is clonal positive.
- There must be ≥ 2 nt difference between two clonal positive peaks.
- Samples should be run in duplicate to confirm both replicates show positive results for a suspected peak.
- The size of the suspected clonal peaks in both replicates must be within ± 1 nt from each other.

7.7. Quality Control

Positive and negative controls are provided with the kit and must be included with each assay run. In addition, a no template control (*e.g.* water) must also be included. A buffer control may also be added to ensure that no contamination of the buffer used to resuspend the samples has occurred. The values for the positive controls are provided under section 10.1 *Expected Size of Amplified Products*. Additional controls and sensitivity controls (dilutions of positive controls into our negative control) are available from Invivoscribe.

7.8. Assay Control

The amplicon sizes listed in Table 4 were determined using an ABI platform. The amplicon sizes measured on your specific capillary electrophoresis instrument may differ by 1 to 4 nucleotides (nt) from those listed depending on the platform of detection and the version of the analysis software used. Once identified, the amplicon size as determined on your specific platform will be consistent from run to run.

Note: "Color" indicates the color of products generated with the master mix when using the default color assignment on ABI fluorescence detection systems.

Table 6. Assay Controls

Master Mix	Target	Color	Control DNA	Cat#	Product Size (nt)	Expected Algorithm Results
***TCRG* - 6FAM**	Vγ1-Vγ11 + Jγ1/Jγ2, JγP, JγP1/JγP2	**Blue**	**Specified Size Range** 5% *TCRG* Positive Control DNA	--- 40883320	**159-207** 194, 196	One peak at 196 nt flagged as "Significant"
Specimen Control Size Ladder	Multiple Genes	**Blue**	*TCRG* Negative Control DNA	40920020	84, 96, 200, 300, 400, 600[a]:	N/A

[a]**Note:** Because smaller PCR fragments are preferentially amplified, it is not unusual for the 600 nt fragment to have a diminished signal or to be missing entirely. For ABI fluorescence detection, the 600 nt peak may not appear during normal run times. In addition, the size of this peak may differ by over 30 nt when fragment size is extrapolated using the GeneScan - 400HD [ROX] size standards.

8. Interpretation of Results

Although positive results are highly suggestive of malignancy, interpret both positive and negative results in the context of all clinical information and laboratory test results. The size range for the *TCRG* – 6FAM master mix has been determined to be 159 nt to 207 nt by testing positive and negative control samples. However, valid clonal *TCR* gamma rearrangements can occur outside the specified size range. Product(s) that are suspect *TCR* gamma gene rearrangement(s) that lie outside the specified size range can be sequenced to confirm their identity.

8.1. Analysis

8.1.1. Report samples that fail to amplify following repeat testing as "**A result cannot be reported on this specimen because the DNA was of insufficient quantity or quality for analysis**".

8.1.2. Repeat testing if the positive or negative control reactions fail.

8.1.3. If samples run in duplicate yield differing results, re-test and/or re-evaluate the samples for sample switching.

8.1.4. **Examine all assay controls prior to interpretation of sample results. If the controls do not yield the correct results, the assay is not valid and the samples must not be interpreted**.

Table 7. The following describes the analysis of each of the controls, and the decisions necessary based upon the results.

Type of Control	Expected Result	Aberrant Result
No Template Control	No amplification present: continue with analysis	Amplification present: check for contamination and repeat the assay.
***TCRG* Negative Control**	Normal Gaussian distribution from 159 nt to 207 nt. No clonal peaks are flagged by Algorithm worksheet. Continue with analysis.	Algorithm worksheet flags one or more peaks as "Significant": Repeat the assay
5% *TCRG* Positive Control (This can also be an extraction control if positive control material is taken through extraction processes)	Peaks present at 194 nt, 196 nt. Algorithm worksheet flags at least the196 nt as "Significant." The 194 nt peak may or may not be identified as "Significant". Continue with analysis.	Algorithm worksheet does not flag peak at 196 nt as "Significant": Repeat the assay.
Specimen Control Size Ladder	If the 100, 200, 300, and 400 nt peaks are observed, continue with analysis. Because smaller PCR fragments are preferentially amplified, it is not unusual for the 600 nt fragment to have a diminished signal or to be missing entirely.	If no peaks are detected, repeat the assay unless specimen tests positive. If only the 100, 200, or 300 nt peaks are present, re- evaluate sample for DNA degradation unless specimen tests positive.

8.2. Sample Interpretation

Given that the controls produce expected results, interpret the clinical samples as follows:

8.2.1. One or two significant peaks flagged by the Algorithm worksheet within the valid size range are reported as "**Positive for the detection of clonal T cell receptor gamma chain gene rearrangement(s) consistent with the presence of a clonal cell population. In the context of overall diagnostic criteria, clonal cell populations can indicate the presence of hematologic malignancy**."

8.2.2. Three or more significant peaks flagged by the Algorithm worksheet within the valid size range are reported as "**T cell receptor gamma chain gene rearrangements are consistent with the detection of biclonality or oligoclonality**."

8.2.3. An absence of significant peaks flagged by the Algorithm worksheet within the valid size range is reported as: "**Negative for the detection of clonal T cell receptor gamma chain gene rearrangement(s)**."

Note: Conduct a visual confirmation to confirm the electropherogram and the algorithm are concordant.

9. Limitations of Procedure

- This assay does not identify 100% of clonal cell populations.
- This assay cannot reliably detect below five positive cells per 100 total cells.
- Always interpret the results of molecular clonality tests in the context of clinical, histological, and immunophenotypic data.
- The algorithm requires a reasonably consistent signal background and that the data is entered correctly. Gaps in the background can cause the algorithm to call a sample incorrectly. Review all electropherograms to confirm the validity of the interpretation.
- PCR-based assays are subject to interference by degradation of DNA or to inhibition of PCR due to EDTA, heparin, or other agents.

10. Expected Values

10.1. Expected Size of Amplified Products

The amplicon sizes listed were determined using an ABI platform. Amplicon sizes seen on your specific capillary electrophoresis instrument may differ 1 to 4 nt from those listed depending on the platform of detection and the version of the analysis software used. Once identified, the amplicon size as determined on your specific platform will be consistent from run to run. This reproducibility is extremely useful when monitoring disease recurrence.

Note: "Color" indicates the color of products generated with the master mix when using the default color assignment on ABI fluorescence detection systems.

Table 8. Expected Size of Amplified Products

Master Mix	Target	Color	Control DNA	Cat#	Product Size (nt)	Expected Algorithm Results
***TCRG*-6FAM**	All V and J genes Vγ9, Vγ10 + Jγ1/Jγ2	**Blue** **Blue**	**Specified Size Range** *TCRG* Negative Control DNA 5% *TCRG* Positive Control DNA	--- 40920020 40883320	**159 - 207** 159 - 207 194, 196	No Significant Peaks Significant Peak at 196 nt and possibly second peak at 194 nt
Specimen Control Size Ladder	Multiple Genes	**Blue**	Any Human DNA	---	84, 96, 200, 300, 400, 600[a]	N/A

[a]**Note :** Because smaller PCR fragments are preferentially amplified, it is not unusual for the 600 nt fragment to have a diminished signal or to be missing entirely. For ABI fluorescence detection the 600 nt peak may not appear during normal run times. In addition, the size of this peak may differ by over 30 nt when fragment size is extrapolated using the GeneScan - 400HD [ROX] size standards.

10.2. Sample Data

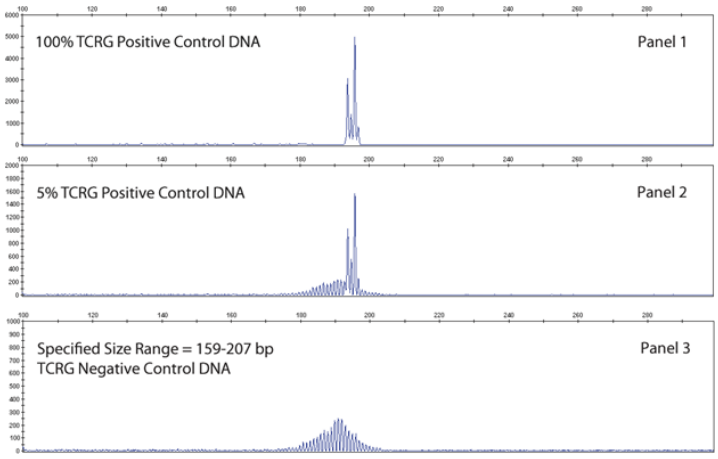

Figure 2. The data was generated using the *TCRG*-6FAM Master Mix. Amplified products were run on an ABI instrument.

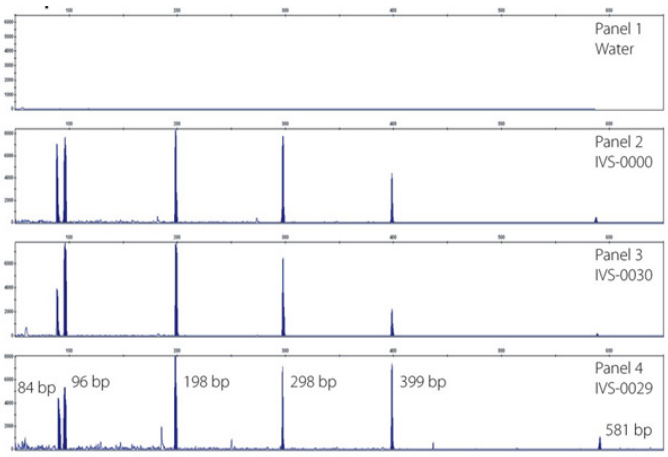

Figure 3. The data was generated using the Specimen Control Size Ladder master mix.

11. Performance Characteristics

The assay was able to detect clonal rearrangements in eleven (11) positive control cell lines.

Table 9. The following well-characterized T-cell leukemia cell lines known to be positive for *TCRG* rearrangements were tested with *TCRG* – 6FAM master mix, and the results are shown below. Two prominent peaks were detected with each of the cell lines.

Cell Line	IVS Part Number	Peak One (nt)	Peak Two (nt)
100% IVS-0004	4-088-0190	178.8	187.9
100% IVS-0005	4-088-0250	173.0	198.3
100% IVS-0008	4-088-0430	195.1	206.7
100% IVS-0009	4-088-0490	187.8	190.5
100% IVS-0016	4-088-0910	169.4	193.7
100% IVS-0021	4-088-1210	183.1	188.0
100% IVS-0039	4-088-2290	193.9	195.9
DND-41	N/A	168.9	188.8
PF-382	N/A	190.6	200.1
MOLT-13	N/A	187.9	190.6

Table 10. The assay showed robust results when tested with IVS-0039 DNA (200 ng/µL) diluted into tonsil DNA (200 ng/µL) at 5%, 10%, 25%, 50% and 75% (v/v).

		Peak One				Peak Two			
		Product Size (nt)	D(x) Value	RPR Ratio	Significant?	Product Size (nt)	D(x) Value	RPR Ratio	Significant?
5% IVS-0039	rep 1	196.20	0.2330	6.04	Yes	194.16	0.1208	2.93	No
	rep 2	195.81	0.1803	5.32	Yes	193.75	0.0993	2.65	No
	rep 3	195.71	0.1872	6.68	Yes	193.65	0.0962	2.45	No
	rep 4	196.18	0.2080	6.34	Yes	194.09	0.0941	2.88	No
	rep 5	195.79	0.1833	6.07	Yes	193.77	0.0931	2.70	No
10% IVS-0039	rep 1	196.24	0.3573	9.76	Yes	194.31	0.2115	5.15	Yes
	rep 2	196.15	0.3382	9.18	Yes	193.95	0.1877	4.03	Yes
	rep 3	195.66	0.2790	8.32	Yes	193.62	0.1819	4.24	Yes
	rep 4	196.15	0.3382	9.18	Yes	193.95	0.1877	4.03	Yes
	rep 5	195.80	0.2983	7.77	Yes	193.75	0.1830	4.69	Yes
25% IVS-0039	rep 1	196.02	0.3947	8.00	Yes	194.16	0.3730	22.2	Yes
	rep 2	196.11	0.3216	6.07	Yes	194.17	0.3568	20.09	Yes
	rep 3	195.72	0.4059	9.39	Yes	193.66	0.3451	17.02	Yes
	rep 4	196.11	0.3212	6.07	Yes	194.17	0.3561	20.09	Yes
	rep 5	195.71	0.4316	10.24	Yes	193.65	0.3482	17.09	Yes
50% IVS-0039	rep 1	196.15	0.2939	4.58	Yes	194.23	0.4545	630.3	Yes
	rep 2	195.67	0.3817	7.31	Yes	193.69	0.4686	120.4	Yes
	rep 3	195.66	0.4503	9.17	Yes	193.62	0.4672	28.15	Yes
	rep 4	196.07	0.3586	5.73	Yes	194.11	0.4607	32.59	Yes
	rep 5	195.67	0.4404	8.85	Yes	193.69	0.4626	124.4	Yes
75% IVS-0039	rep 1	196.11	0.2387	3.36	No	193.89	0.3532	58.40	Yes
	rep 2	195.72	0.3154	5.29	Yes	193.66	0.4799	71.81	Yes
	rep 3	195.62	0.4520	9.16	Yes	193.57	0.4811	110.9	Yes
	rep 4	196.15	0.2911	4.45	Yes	194.14	0.4387	79.15	Yes
	rep 5	195.71	0.3301	5.86	Yes	193.65	0.4708	78.47	Yes

Table 11. The assay, when performed in combination with the *TCRG* Algorithm worksheet, was capable of detecting DNA from 6 control cell lines (200 ng/µL) diluted into tonsil DNA (200 ng/µL) at 5% (v/v) and the results are shown below.

		Peak One				Peak Two			
		Product Size (nt)	D(x)	RPR Ratio	Significant?	Product Size (nt)	D(x)	RPR Ratio	Significant?
5% IVS-0004	rep 1	178.87	0.2964	42.50	Yes	184.69	0.1322	5.61	Yes
	rep 2	184.36	0.1193	27.59	Yes	178.39	0.0908	21.13	Yes
	rep 3	184.32	0.1266	22.96	Yes	178.32	0.1041	23.93	Yes
	rep 4	184.69	0.1200	14.97	Yes	178.78	0.1009	29.82	Yes
	rep 5	184.36	0.1342	12.12	Yes	178.40	0.1146	32.07	Yes
5% IVS-0016	rep 1	169.38	0.1035	115.9	Yes	193.71	0.1016	2.32	No
	rep 2	169.03	0.0918	159.0	Yes	193.50	0.0857	2.24	No
	rep 3	168.99	0.0975	55.00	Yes	193.41	0.0791	2.25	No
	rep 4	169.38	0.1028	100.9	Yes	193.77	0.1041	2.45	No
	rep 5	169.00	0.0957	55.0	Yes	193.53	0.0944	2.50	No
5% IVS-0021	rep 1	187.91	0.1120	7.28	Yes	182.92	0.1239	14.57	Yes
	rep 2	187.58	0.1003	5.67	Yes	182.53	0.1298	14.23	Yes
	rep 3	182.50	0.0950	35.5	Yes	187.50	0.1110	5.54	Yes
	rep 4	187.92	0.1112	6.99	Yes	183.01	0.1238	12.68	Yes
	rep 5	187.62	0.0978	6.09	Yes	182.67	0.1253	24.69	Yes
5% IVS-0039	rep 1	195.97	0.2907	8.46	Yes	193.95	0.1576	3.60	No
	rep 2	195.70	0.2221	6.74	Yes	193.59	0.1321	3.04	No
	rep 3	195.56	0.2010	6.86	Yes	193.53	0.1244	2.84	No
	rep 4	196.01	0.2942	8.05	Yes	194.01	0.1484	3.18	No
	rep 5	195.71	0.2513	7.53	Yes	193.65	0.1470	3.36	No
5% PF-382	rep 1	191.84	0.2784	5.77	Yes	158.41	0.3057	123.5	Yes
	rep 2	191.48	0.2558	5.30	Yes	158.24	0.2739	125.7	Yes
	rep 3	191.57	0.2418	5.44	Yes	158.15	0.2787	115.1	Yes
	rep 4	191.84	0.2822	5.68	Yes	158.33	0.2811	118.8	Yes
	rep 5	191.55	0.2524	5.63	Yes	158.15	0.2883	93.0	Yes
5% MOLT-13	rep 1	190.74	0.2147	3.97	No	187.92	0.1292	6.96	Yes
	rep 2	190.46	0.1806	3.51	No	187.60	0.1081	4.96	Yes
	rep 3	190.46	0.1731	3.42	No	187.53	0.1039	5.27	Yes
	rep 4	190.64	0.2132	4.10	Yes	187.93	0.1215	5.69	Yes
	rep 5	190.46	0.1983	6.19	Yes	187.57	0.1114	5.80	Yes

Note: 5% IVS-0004 is REF 4-088-0230, 5% IVS-0016 is REF 4-088-0950, 5% IVS-0021 is REF 4-088-1250 and 5% IVS-0039 is REF 4-088-2330.

Using clinical samples, the *TCRG* V2 assay results were compared to Roche 454 sequencing for the identification T-cell receptor gamma gene rearrangements. For the 454 sequencing, any DNA sequence that was present at levels greater than 5% of the total sequences detected was considered a clonal event. If more than two (2) sequences exceeded the 5% threshold, that sample was defined as oligoclonal. The *TCRG* V2 assay had 100% concordance for the seven (7) samples that were identified as clonal by sequencing. There was 75% concordance for the twelve (12) samples that were either negative for a clonal event or were oligoclonal. Sample types included peripheral blood, bone marrow, and FFPE samples. It is important to note that the presence or absence of clonal peaks in a clinical sample does not always correlate with actual clinical outcomes.

12. Technical and Customer Service

Technical and Customer Service Representatives are available Monday through Friday to answer phone, e-mail or website inquiries.

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13. Bibliography

1. Miller, JE, et al., An automated semiquantitative B and T cell clonality assay. *Mol. Diag.* 1999, 4(2):101-117.

14. Symbols

The following symbols are used in labeling for Invivoscribe products.

IVD	For *In Vitro* Diagnostic Use		Expiration Date
REF	Catalog Number	EC REP	Authorized Representative in the European Community
VOL	Reagent Volume		Manufacturer
LOT	Lot Number		Consult Instructions for Use
	Storage Conditions		

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